polytetrafluoroethylene (Teflon), or equivalently permeable material, permits oxygen enrichment of a nutrient medium to an extent which makes possible maximum propagation of insect cells in suspension volumes of ten liters or more. The cells are not prone to settle on, or attach to, the permeable tube **4** and thus do not slowly block oxygen flow, because the material of which the tubes are made provides a surface not suitable for cell adherence. For the same reason, cells do not adhere between the tube spirals, die there, decay and contaminate the nutrient medium with toxic decay products.

The following examples are presented to further illustrate the invention.

EXAMPLE 1

A suspension culture was started from the insect cell line IZD-Mb 0503 (IZD=Institute for Zoology Darmstadt; Mb=Mamestre brassicae=a type of butterfly; 0503=code number of the cell line; Lepidoptern-cell-line ATCC #CRL 8003) in a so-called spinner container, to which a standard liquid nutrient medium (pH 6.6) was added, with constant stirring by means of a magnetic stirrer. The nutrient medium used was published by T. D. Grace in Nature, 195, 788–789 (1962). The cells were permitted to multiply freely suspended in the nutrient medium. A starting population of 2×10^5 cells/ml of nutrient medium is necessary for multiplication. After three days, as a rule, a cell population of about 6 to 10×10^5 cells/ml is obtained. This is a population increase of 3 to 5 times the original amount. Because of nutrient depletion and "aging" of this culture, even with a longer fermentation time, a higher cell population cannot be achieved. This "parent culture" provides the cell preparation used to start a cell culture in a fermenter.

The oxygen and pH measuring electrodes of a fermenter like that shown in FIG. **1**, were calibrated, autoclaved and recalibrated. After that they were inserted in the fermenter cover. A tube of silicone rubber having a 1.0 mm wall thickness was used as the membrane and wound on the heat exchanger. The cover (with the electrodes) and all parts of the fermenter which contact the cell suspension and the nutrient medium (same as above) flowing back and forth, as well as the systems supplying and removing the airstream, are sterilized in an autoclave.

The fermenter vessel was put together observing all conditions necessary to maintain the equipment sterile. The desired nutrient medium volume was filled through openings in the fermenter vessel cover provided for this purpose. Two liters of nutrient medium, to which cells were added to provide a population of 10^5 cells/ml, were added to the 12 liter capacity fermenter vessel.

The fermenter was put into operation with stirring at 60 to 70 RPM, and a temperature of 28° C. in the suspension. The initial oxygen value (7.5 mg/l) and pH value were set via the measuring electrodes.

In the first 16 to 24 hrs. after the start of the cultivation, an oxygen enrichment of the nutrient was not absolutely necessary. However, oxygen enrichment is needed when the cell culture enters into its logarithmic growth phase and when the oxygen in the nutrient is used up due to the increase of the cell population and increased metabolism.

The oxygen is supplied via the silicone tube functioning as a permeable membrane through which oxygen from atmospheric air, or from an oxygen-air mixture, diffuses into the nutrient medium. To enrich the nutrient medium after the first 16 to 24 hrs., compressed air at a pressure of 0.5 to 1.0 atmosphere (gauge) was introduced via the silicone tube. If necessary, the pressure can be increased up to 2.0 atmospheres.

With an initial cell concentration of 10^5 ml, the oxygen concentration decreases from 7.5 mg/l within 24 hours during the logarithmic cell increase (cell proliferation) to below 1% of the initial value. However, by means of oxygen (air) supplied through the silicone tube during the logarithmic cell increase, the oxygen concentration can be maintained at 10 to 30% of the initial concentration, which guarantees very good cell proliferation.

By means of the oxygen diffusion method according to the invention the number of cells per ml of nutrient medium was increased from 10^5 up to 2 to 3×10^6 in four days.

After 2 to 3 days, maximum cell multiplication has been surpassed and the cell culture has entered the stationary phase in which the cells gradually stop dividing. Each ml then contains 2 to 3×10^6 cells. Then 6 to 7 liters of fresh nutrient medium was added under sterile conditions. The cell concentration was correspondingly reduced. The cells then change over from the stationary phase into a multiplying phase. After 2 to 3 days fermentation a cell concentration of 2 to 3×10^6 ml was again obtained. From the original 3×10^8 cells/3 liters, up to about 10^{11} cells have developed in a total volume of 10 liters.

Using the bubbling air method of the prior art, a volume increase of more than 4 liters would not have been possible. Four prior art cell propagations of 2.5 liters each would have resulted, at the most, in about 4×10^9 cells. The membrane method according to the invention yields a 20 times higher cell population.

About 5 liters of the cell suspension was removed from the 10 liter volume when the stationary phase was reached (2nd to 3rd day). Then 5 liters of fresh nutrient medium was added under sterile conditions to the fermenter. Thus, the remaining suspension was diluted and the cells, due to the new nutrient supply, again started their multiplying phase. Repetitions of these diluting-multiplying phases, when sterile conditions are maintained in the fermenter vessel, can be continued as long as permitted by the total condition of the cells. In stabilized cell lines, this can lead to a continuous operation.

EXAMPLE 2

The following cell lines can be fermented using the method described in example 1:

IZD-Mb 2006=Mamestra brassicae
IZD-Mb 1203=Mamestra brassicae
IZD-Mb 0504=Mamestra brassicae
IZD-Ld 1307=Lymantria dispar
IZD-Ld 1407=Lymantria dispar

A multiplication rate, equally as good as with IZD-Mb 0503, can be expected for all of the listed insect cell lines. Other cell lines in addition to those just listed, which grow as suspension cultures, can be propagated or multiplied by the membrane oxygen diffusion method of the invention.

EXAMPLE 3

Recently it has become possible to propagate cells, not previously multipliable as a culture suspension, in a fermenter. Oxygen was supplied by bubbling air. By the use of so-called micro-carriers which are, as a rule,